

attached to the N-terminus of the mature hSDF-1 $\alpha$  or hSDF-1 $\beta$  protein; these proteins are referred to as GroHEK/hSDF-1 $\alpha$  and GroHEK/hSDF-1 $\beta$  and have the amino acid sequences shown in SEQ ID NO:12 and SEQ ID NO:13, respectively. The expression vectors containing the hSDF-1 PCR products were sequenced and used to transform the *E. coli* strain GI934 (Lu *et al.*, 1996, *J. Biol. Chem.* 271: 5059-5065). The resulting transformed strains hSDF-1 $\alpha$ , hSDF-1 $\beta$ , GroHEK/hSDF-1 $\alpha$ , and GroHEK/hSDF-1 $\beta$  were deposited with the American Type Culture Collection, P.O. Box 1549, Manassas, Virginia 20108 (previously located at 12301 Parklawn Drive, Rockville, Maryland 20852) on August 15, 1997 and were given the accession number ATCC 98506, ATCC 98507, ATCC 98508, and ATCC 98509, respectively.

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**REMARKS**

The Specification has been amended to insert the location of the American Type Culture Collection at line 5 on page 43, pursuant to the requirement of 37 C.F.R. 1.809(d)(4). Applicants submit that no new matter has been added as a result of the amendment. Pursuant to 37 C.F.R. 1.121(b), Appendix A at pp. 5-6 of this paper provides a marked-up copy of the paragraph amended in the Specification.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 10-0447 (Reference No.: 50657-05302USP1).

Respectfully submitted,

JENKENS & GILCHRIST,  
A Professional Corporation



Lekha Gopalakrishnan  
Reg. No.: 46,733

Date: February 22, 2002

JENKENS & GILCHRIST  
A Professional Corporation  
1445 Ross Avenue, Suite 3200  
Dallas, Texas 75202  
(214) 965-7364  
(214) 855-4300 (fax)

## APPENDIX A

### EXAMPLE 1 - EXPRESSION AND PURIFICATION OF N-TERMINAL-MODIFIED CHEMOKINES

The amino acid sequences of the full-length human chemokines SDF-1 $\alpha$  and SDF-1 $\beta$  (hSDF-1 $\alpha$  and hSDF-1 $\beta$ , GenSeq accession numbers R75419 and R75420) are provided as SEQ ID NO:s 1 and 2, respectively, and SEQ ID NO:s 3 and 4 are the nucleotide sequences of cDNA molecules encoding hSDF-1 $\alpha$  and hSDF-1 $\beta$  (GeneSeq accession numbers Q74089 and Q74091). The amino acid sequences of the mature hSDF-1 $\alpha$  and hSDF-1 $\beta$  proteins begin at amino acid 22 (lysine) in both SEQ ID NO:1 and SEQ ID NO:2. Polymerase chain reaction (PCR) with hSDF-1 $\alpha$  or hSDF-1 $\beta$  cDNA as a template was used to make expression constructs encoding mature hSDF-1 $\alpha$  and hSDF-1 $\beta$  proteins, or mature hSDF-1 $\alpha$  and hSDF-1 $\beta$  proteins fused to the C-terminus of an expression/purification accessory sequence such as GroHEK (SEQ ID NO:5, AAKDVKHHHHHGSGSDDDDK). Cloning NdeI/XbaI-restricted hSDF-1 $\alpha$ , hSDF-1 $\beta$ , GroHEK/hSDF-1 $\alpha$ , and GroHEK/hSDF-1 $\beta$  PCR products (generally referred to as the hSDF-1 PCR products) into the *E. coli* expression vector pAL781 (LaVallie *et al.*, 1993, *Biotechnology (NY)* 11: 187-193) fused the hSDF-1 PCR products in-frame to an ATG codon which serves as the translation initiation codon, producing the four coding sequences shown as SEQ ID NO:6 - SEQ ID NO:9. When hSDF-1 $\alpha$  and hSDF-1 $\beta$  are expressed from these vectors, the resulting proteins have a methionine residue attached to the N-terminus of the mature hSDF-1 $\alpha$  or hSDF-1 $\beta$  protein; these proteins are referred to as met-hSDF-1 $\alpha$  and met-hSDF-1 $\beta$  and have the amino acid sequences shown in SEQ ID NO:10 and SEQ ID NO:11, respectively. Similarly, when GroHEK/hSDF-1 $\alpha$  and Gro

HEK/hSDF-1 $\beta$  are expressed from these vectors, the resulting proteins have the GroHEK peptide attached to the N-terminus of the mature hSDF-1 $\alpha$  or hSDF-1 $\beta$  protein; these proteins are referred to as GroHEK/hSDF-1 $\alpha$  and GroHEK/hSDF-1 $\beta$  and have the amino acid sequences shown in SEQ ID NO:12 and SEQ ID NO:13, respectively. The expression vectors containing the hSDF-1 PCR products were sequenced and used to transform the *E. coli* strain GI934 (Lu *et al.*, 1996, *J. Biol. Chem.* 271: 5059-5065). The resulting transformed strains hSDF-1 $\alpha$ , hSDF-1 $\beta$ , GroHEK/hSDF-1 $\alpha$ , and GroHEK/hSDF-1 $\beta$  were deposited with the American Type Culture Collection P.O. Box 1549, Manassas, Virginia 20108 (previously located at 12301 Parklawn Drive, Rockville, Maryland 20852) on August 15, 1997 and were given the accession number ATCC 98506, ATCC 98507, ATCC 98508, and ATCC 98509, respectively.